Reduction of Various Urinary Metabolites of Tobacco Toxins in Smokers who Switched from Conventional Light Cigarettes to a Cigarette with Low Levels of Tobacco-Specific Nitrosamines and a Modified Filter Tip

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Key Questions

- **Does reduction of select toxic constituents in mainstream smoke (MS) influence metabolic activation and detoxification pathways of other classes of carcinogens?**
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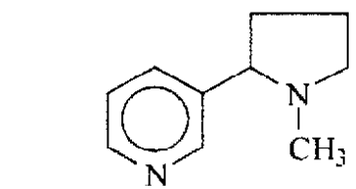
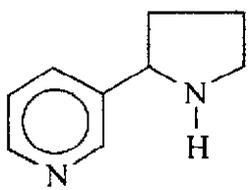
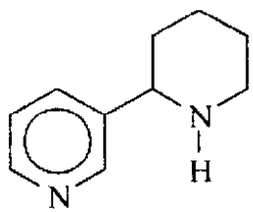
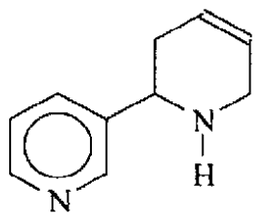
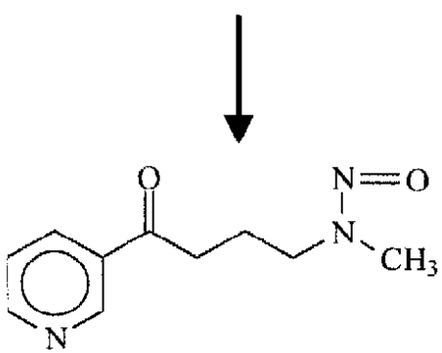
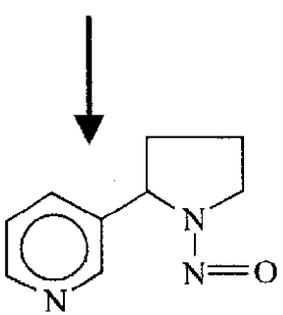
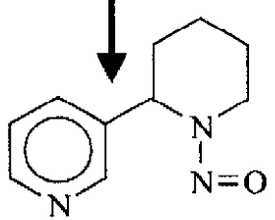
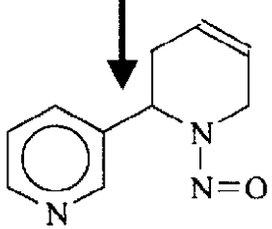
Tobacco Specific Nitrosamines (TSNA)

Nicotine	Nornicotine	Anabasine	Anatabine
			
			
NNK*	**NNN ***	**NAB**	**NAT**
Reduced levels in Test Cig.	**80%**	**74%**	**54%**	**43%**

***Considered Carcinogenic by IARC**

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Reduced Levels of Toxic Constituents in Test Cigarette in Comparison to the two Top-Selling Brands of Cigarettes[a]

Chemical	Percent Reduction
TSNA	43-80 %
Volatile Carbonyls	30-49 %
Volatiles	14-55 %
Toxic trace metals	
[illegible]	[illegible]
[illegible]	[illegible]
[illegible]	[illegible]
Other:	
Ammonia	79 %
Benzo(a)pyrene	Not statistically different
Carbon monoxide	19 %
Hydrogen cyanide	62 %
Nitric oxide	57 %

[a]Data from Test Cigarette Package, (FTC method)

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URINARY METABOLITES TESTED as BIOMARKERS of EXPOSURE

NNAL for TSNA

Benzene metabolites for Volatiles

1-OH-Pyrene for PAH

Thiocyanate for Hydrogen cyanide

cotinine for Nicotine

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Key Questions

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- **Are the urinary metabolites of carcinogens (after 4 weeks exposure) efficient biomarkers?**

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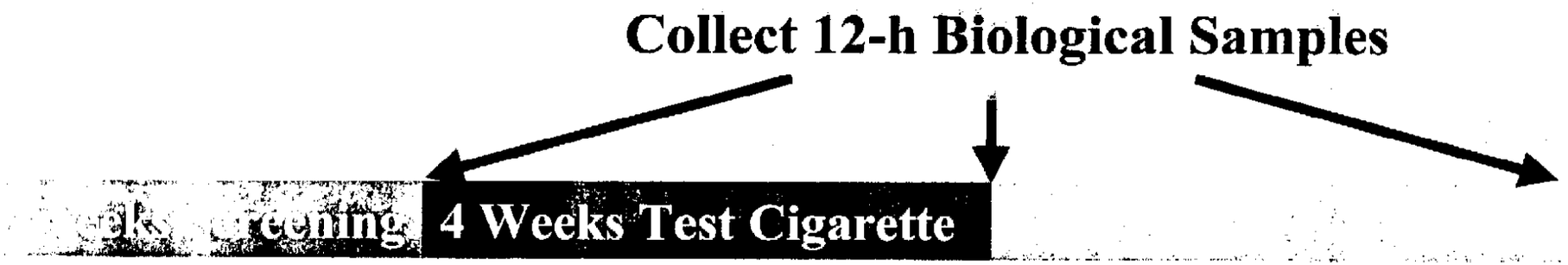
Study Subjects

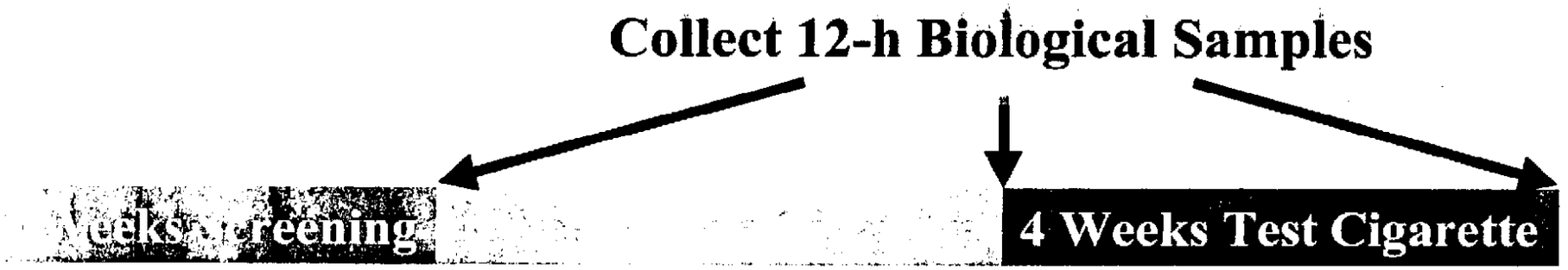
- Number of Subjects: Healthy 20 men, 13 women.
- Age: 21 to 55 years
- Type and Number of Cigarettes Smoked: Smoked ≥20 non-menthol cigarettes per day with 0.8-1.0 mg nicotine/cigarette (FTC method). Agree to not use cigarette brands other than those offered
- Addiction: Fagerström score was ≥6
- Dietary restriction: Subjects were asked to avoid eating charcoal-broiled, smoked or grilled foods and preserved meats to minimize uptake of nitrosamines and polycyclic aromatic hydrocarbons (PAHs) from these sources

Study Design

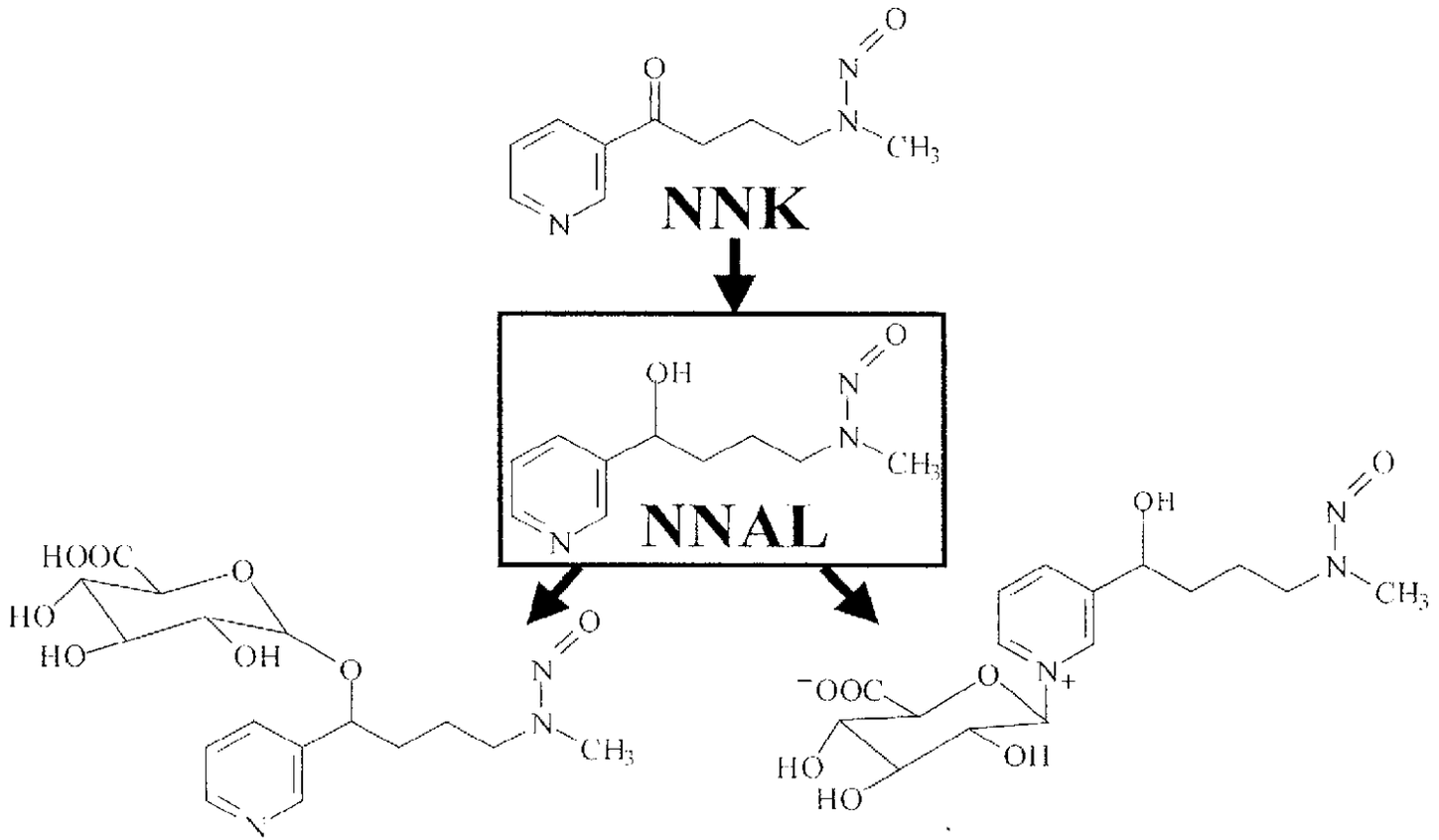
Group A (10 men, 7 women)



Group B (10 men, 6 women)



Metabolic Activation and Detoxification of NNK



Method of Analysis: GC-TEA

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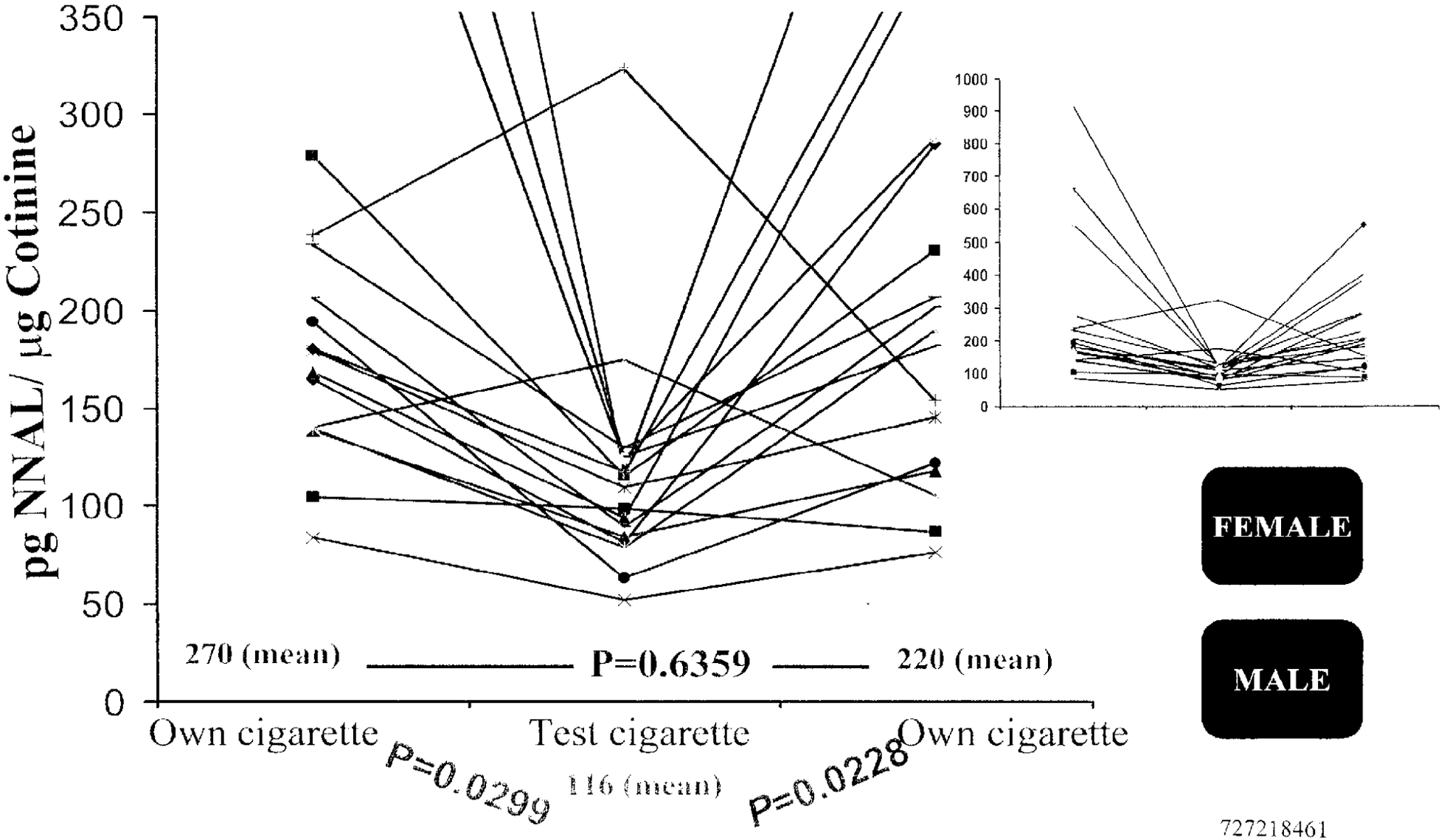
Levels of Urinary NNAL in Group A



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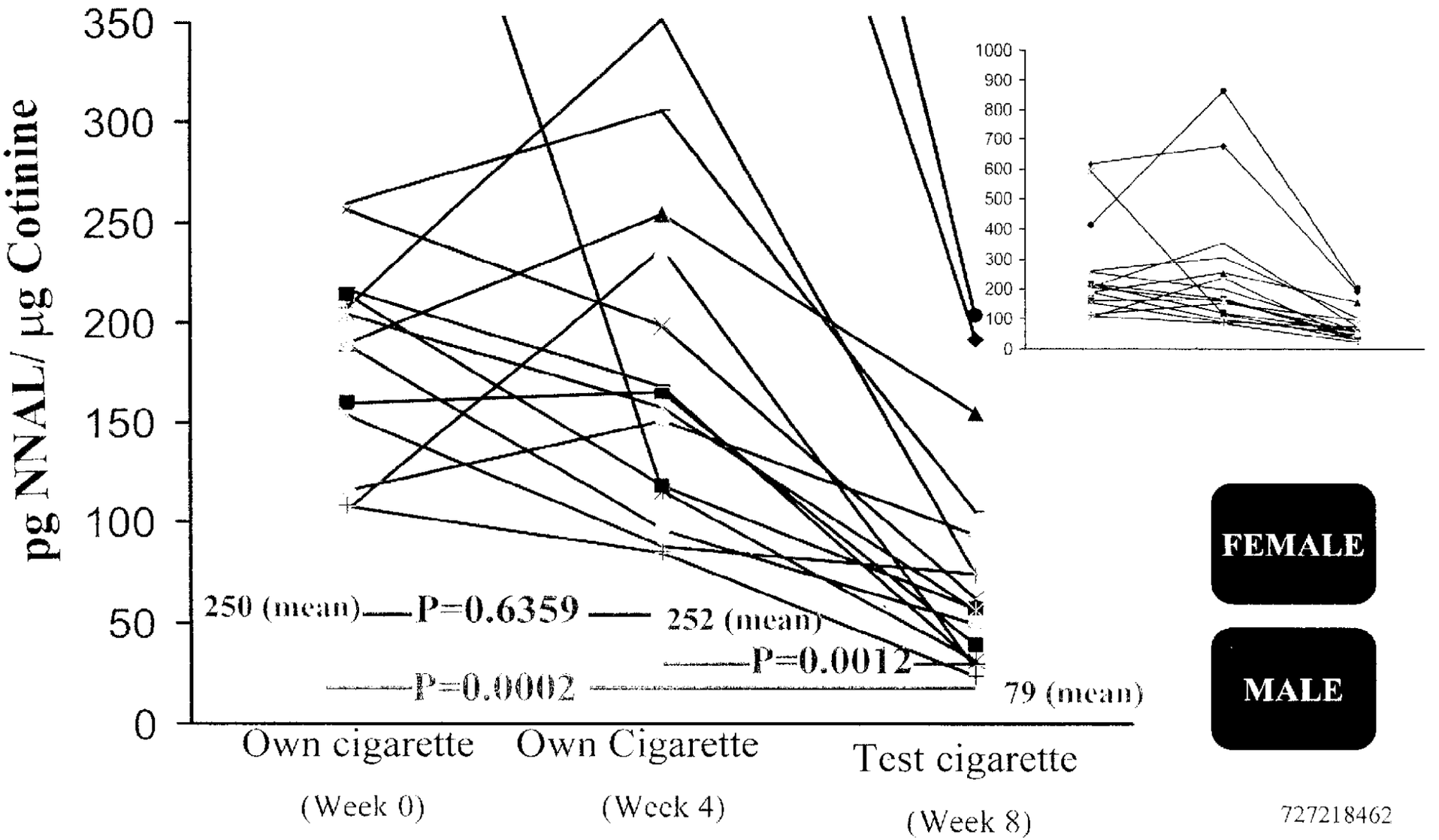
Levels of Urinary NNAL in Group B



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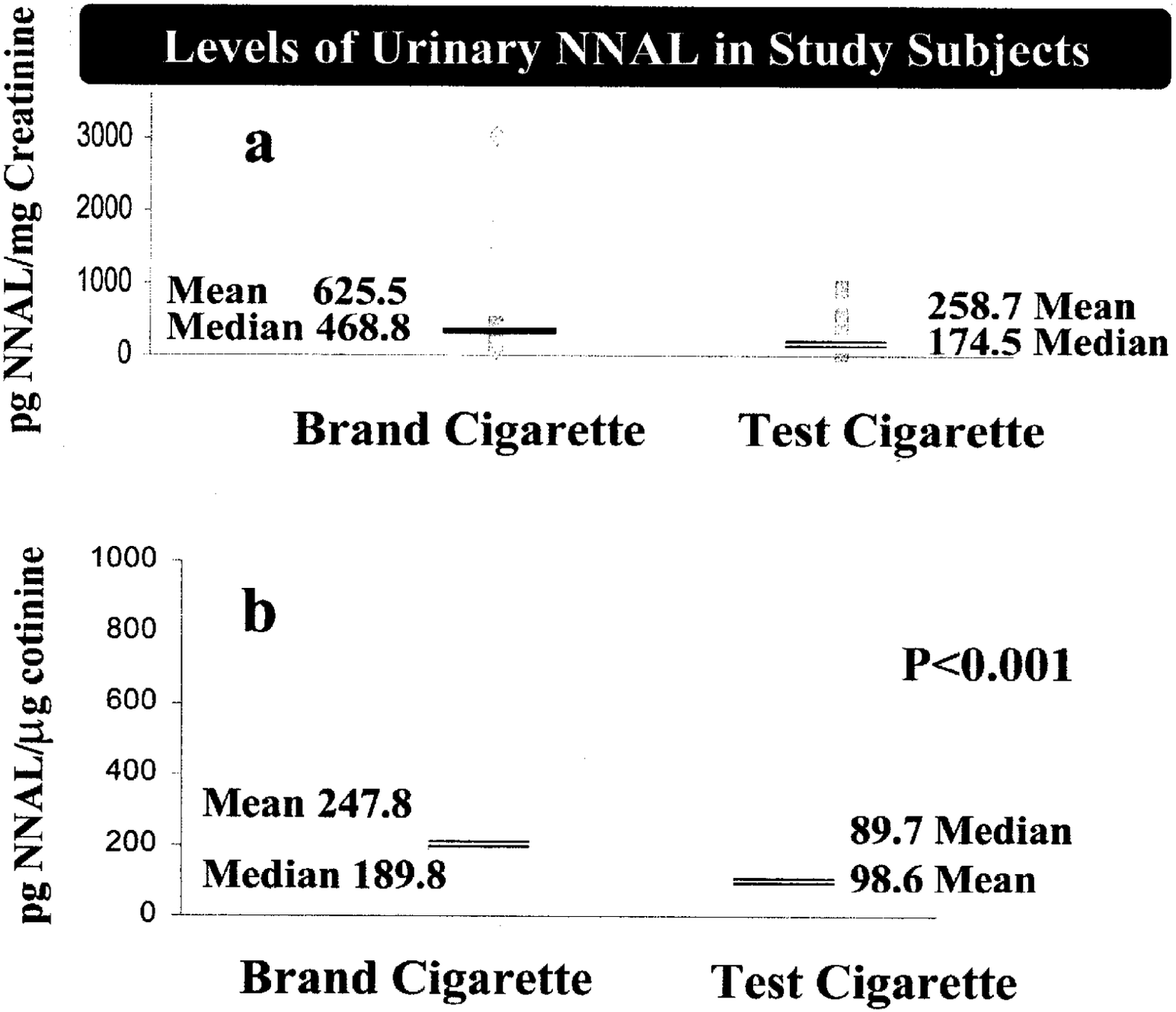
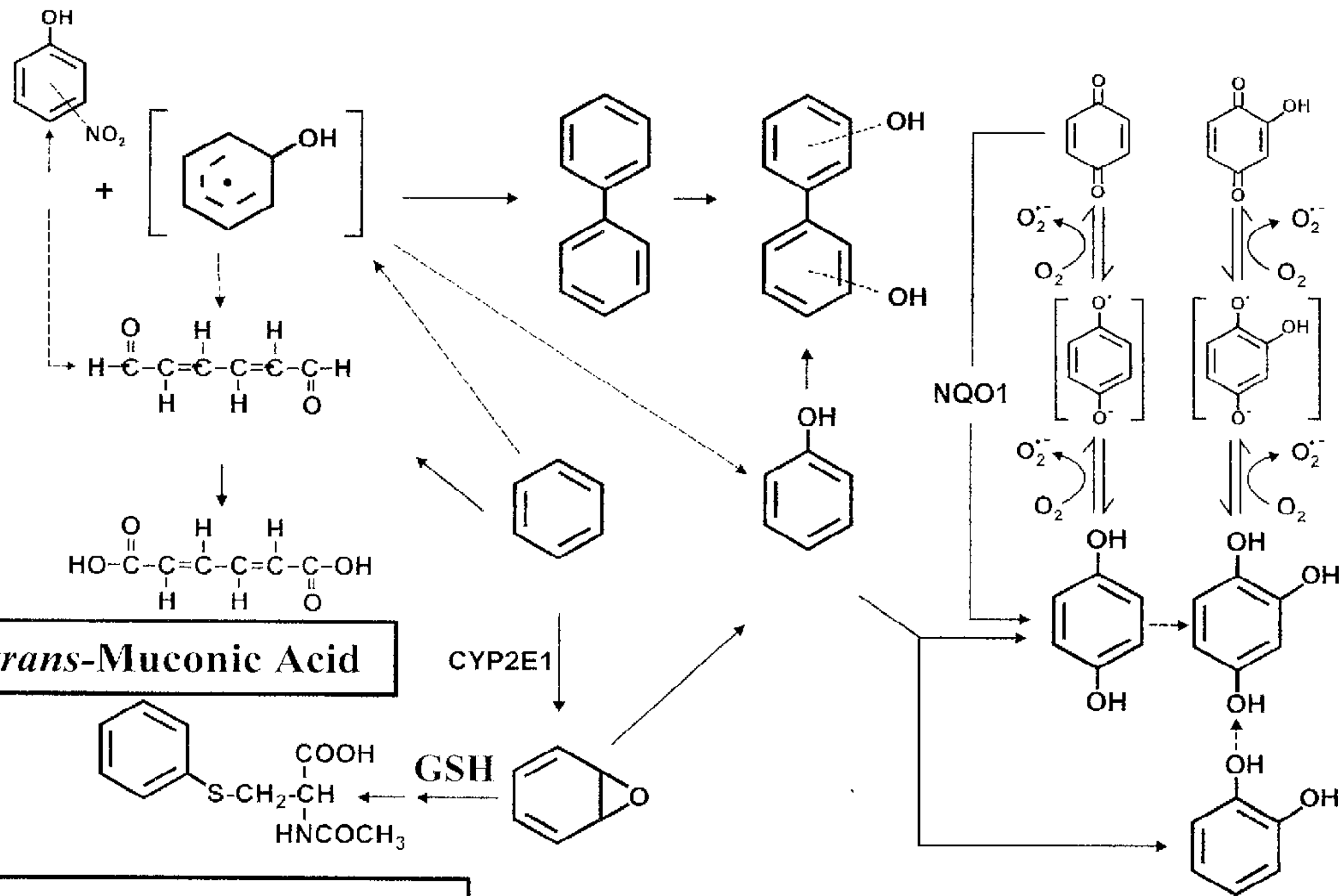
Levels of Urinary NNAL in Study Subjects



a: Adjusted for Urinary creatinine b: Adjusted for creatinine and cotinine

Metabolic Activation Pathways of Benzene



trans,trans-Muconic Acid

S-Phenylmercapturic acid

Method of Analysis: LC-MS/MS

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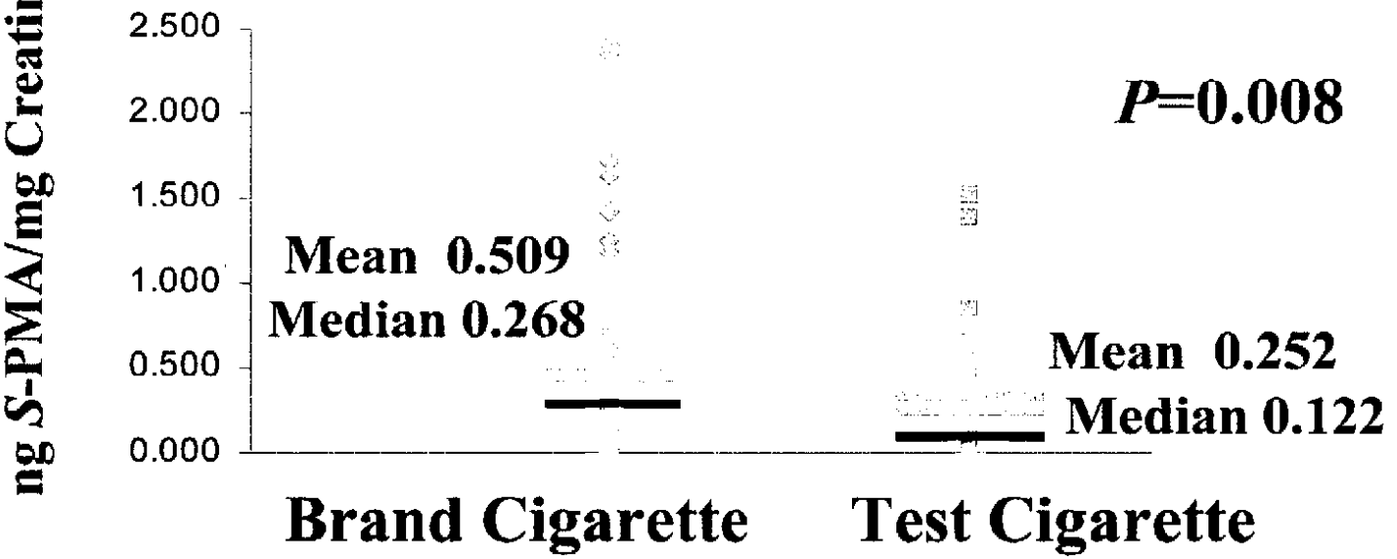
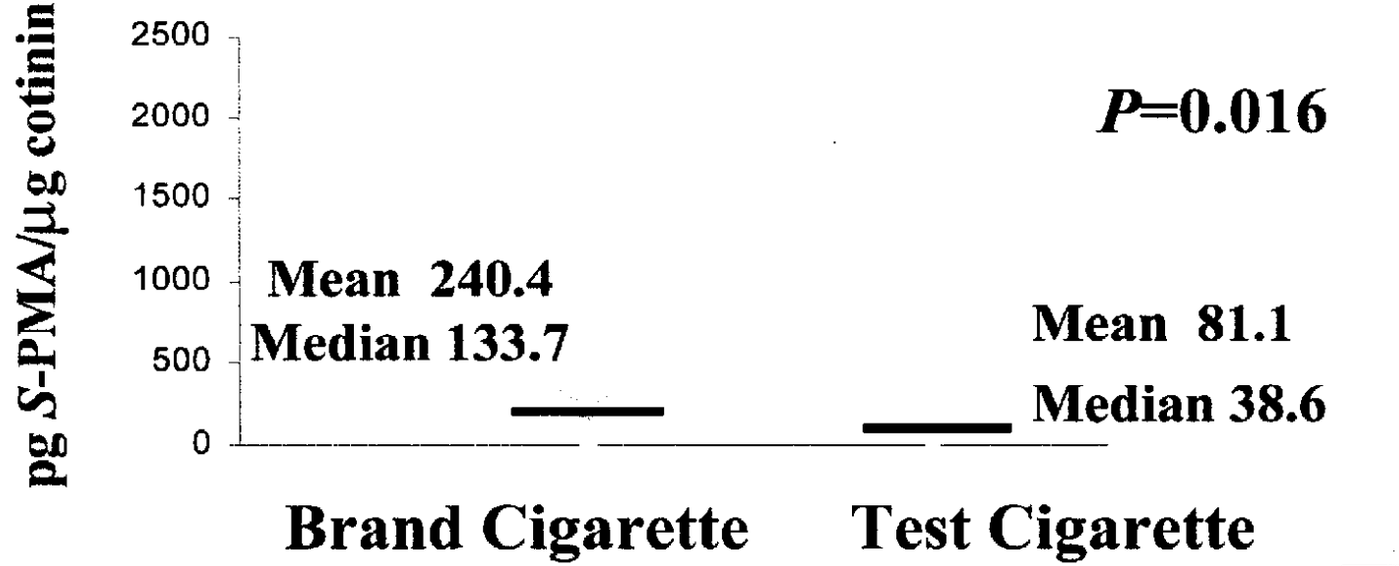
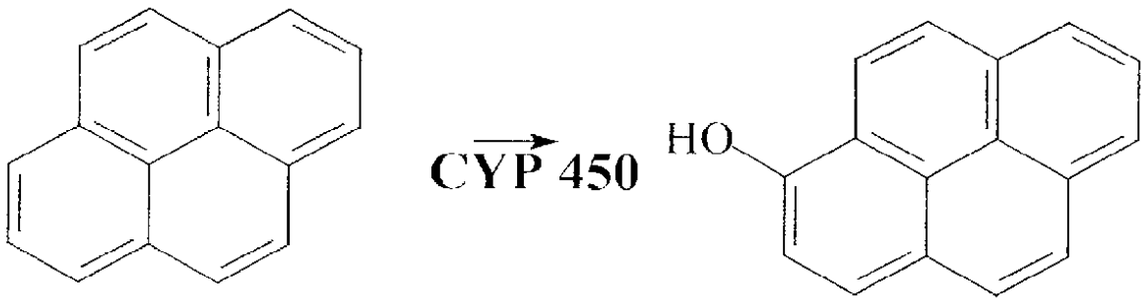
Levels of Urinary *S*-PMA in Study Subjects



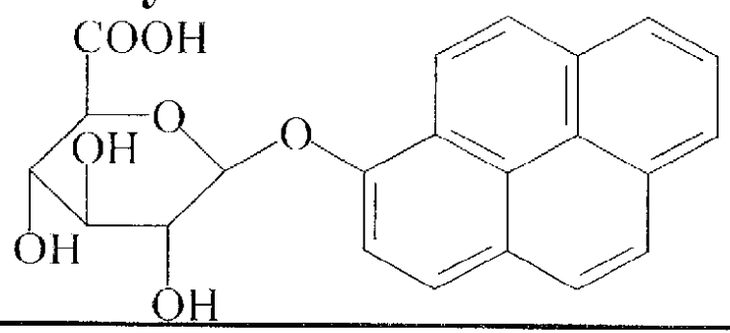


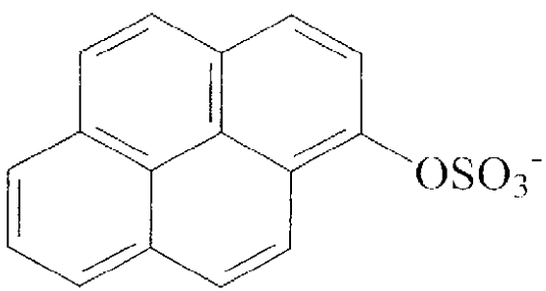
Metabolic Activation and Detoxification of Pyrene



1-Hydroxypyrene (1-OH-P)

Pyrene





1-Pyrenyl-β-glucopyranosiduronic acid (1-OH-P-Gluc) excreted in urine

Pyrene-1-Sulfate (1-OH-P-Sul) excreted in urine

Method of Analysis: HPLC-Fluorescence Detection

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Comparison of Means of Urinary Metabolites

	NNAL	*S*-PMA	*t,t*-MA	1-OH-P	Thiocyanate	Cotinine
Own Cigarette[a]	625.5[c]	0.509[c]	427.7	0.477[c]	0.315	2679
n=66	(ng/g)	(μ g/g)	(μg/g)	(μg/g)	(μM/mg)	(μg/g)
Test Cigarette[a]	258.7[c]	0.252[c]	311	0.377[c]	0.276	2939
n=33	(ng/g)	(μ g/g)	(μg/g)	(μg/g)	(μM/mg)	(μg/g)
Own Cigarette[b]	247.8[c]	240.4[c]	225.7	0.229[c]	0.143	
n=66	(pg/ μg)	(μ μg)	(ng/μg)	(ng/μg)	(μM/μg)	
Test Cigarette[b]	98.6[c]	81.1[c]	181.1	0.139[c]	0.114	
n=33	(pg/μg)	(μ g)	(ng/μg)	(ng/μg)	(μM/μg)	

a) Adjusted for urinary creatinine b) Adjusted for creatinine and cotinine c) $p<0.05$

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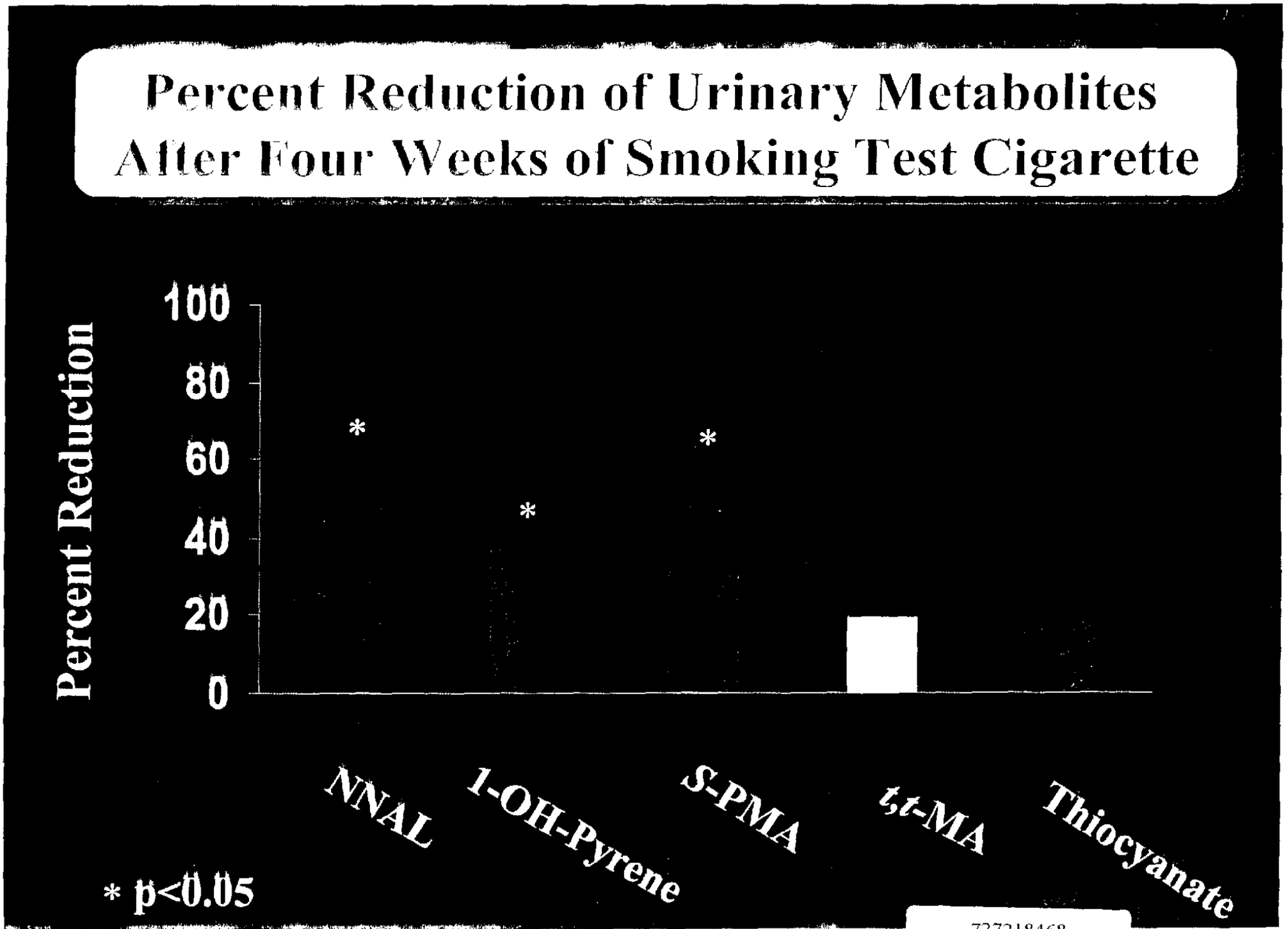
Percent Reduction of Urinary Metabolites After Four Weeks of Smoking Test Cigarette



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Summary

Levels of urinary metabolites are reduced after four weeks of switching to test cigarettes:

(Biological sample)		(Cigarette Smoke)
• NNAL	↓60%	(NNK ↓ 80% in cigarette)
• 1-hydroxypyrene	↓40 %	(pyrene ↓?)
• *S*-PMA	↓ 52%	(benzene ↓ 38%)
• *t,t*-MA	↓20%	(benzene ↓ 38%)
• thiocyanate	↓20%	(hydrogen cyanide ↓ 62%)

The larger reduction for all analytes was observed for the smokers in group B who switched from their usual cigarette to the test cigarette after 4 weeks. NNAL was reduced by 70%, 1-OH-P by 55%, *S*-PMA 73%, *t,t*-MA 64% and thiocyanate by 40%.

Conclusions:

- New Test Cigarette decreased significantly uptake and metabolism of TSNA, BENZEN, and PAH and did not increase HCN metabolism in smokers.
- Urinary metabolites (after 4 weeks of smoking test cigarettes) are efficient biomarkers for exposure.

Acknowledgments: Supported by Star Scientific, Inc.

Urinary Metabolite of Analytes Quantified in Smokers

Estimated Toxic Smoke Constituents	Top 2 selling Brand Cigarettes	Test Cigarette[a]	% Change
TSNA (ng/cig):			
NNK*	95	19	80% ↓
Volatiles: (μg/cig)			
Benzene*	38.6	24.1	38% ↓
Other :			
Pyrene for PAHs			
Benzo(a)pyrene* (ng/cig)	6.6	6.7	NSD
Hydrogen Cyanide (μg/cig)	83.2	31.6	62% ↓

[a]Data from Test Cigarette Package

*Considered Carcinogenic by IARC

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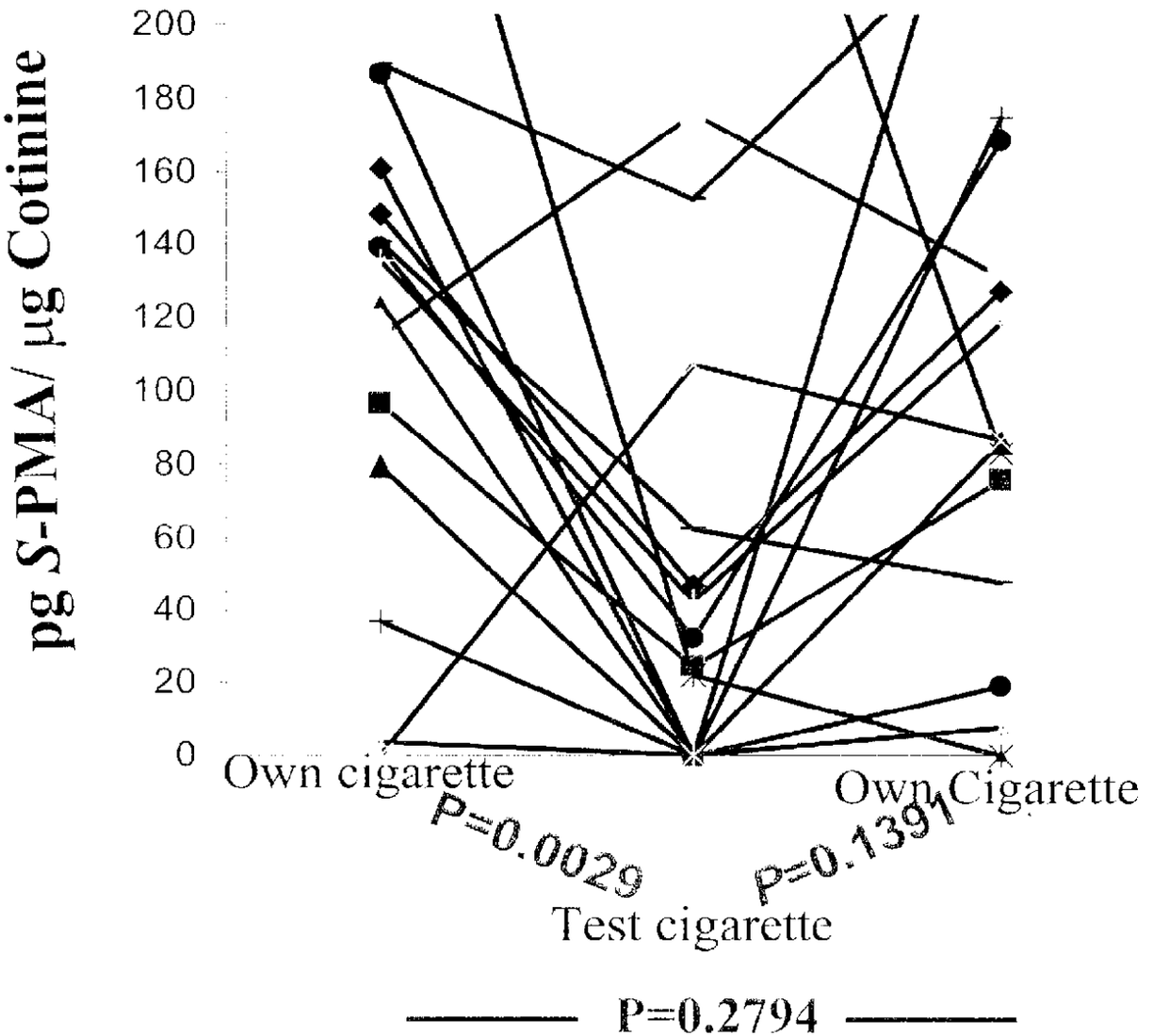
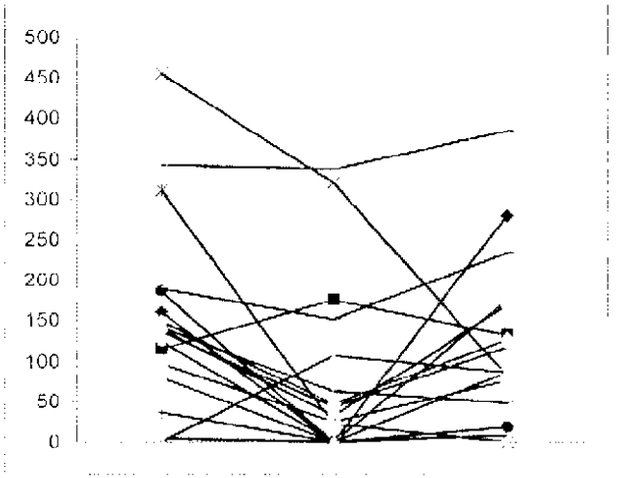
Levels of Urinary *S*-PMA in Group A





FEMALE

MALE

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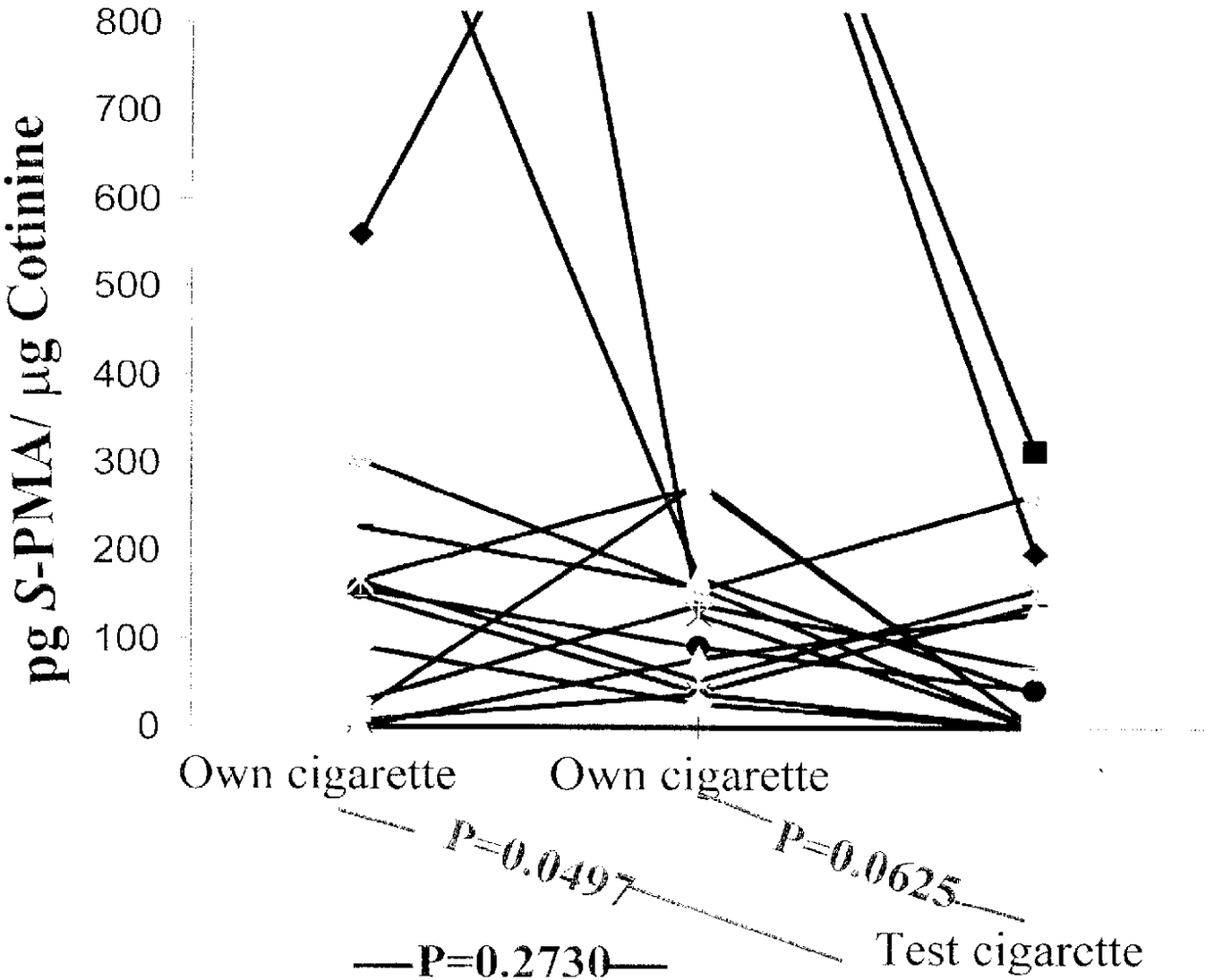
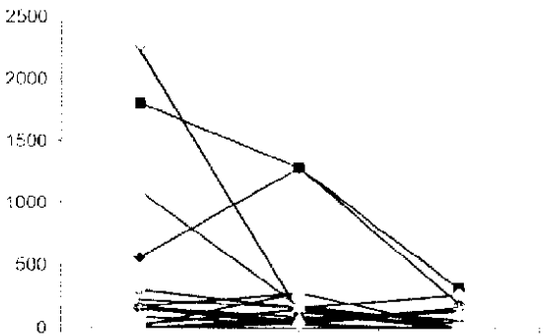
Levels of Urinary *S*-PMA in Group B





FEMALE

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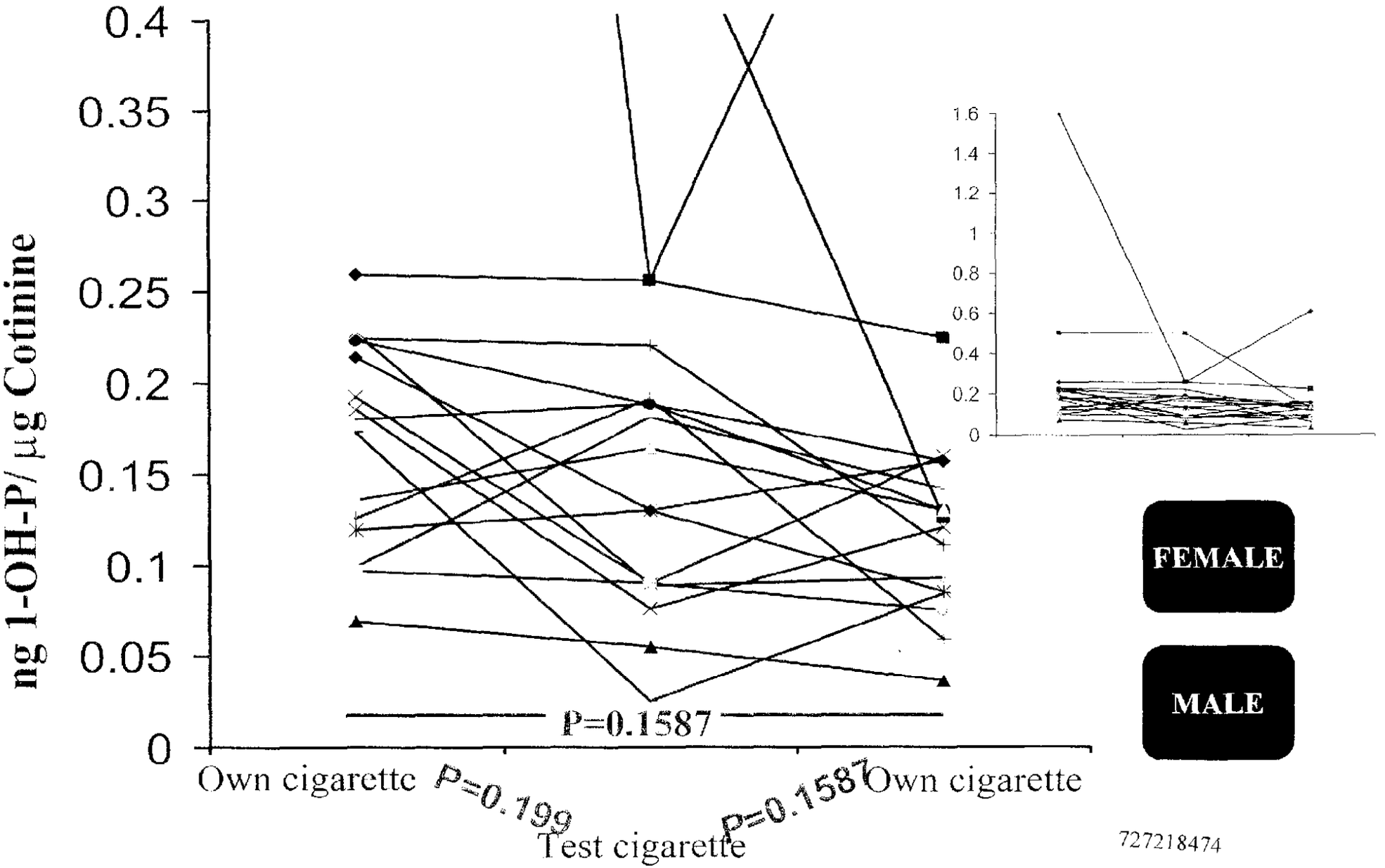
Levels of Urinary 1-Hydroxy-Pyrene in Group A



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Levels of Urinary 1-Hydroxy-Pyrene in Group B